Embryonic Velar Structure and Function of Two Sibling Species of *Crepidula* With Different Modes of Development

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Abstract. The structure and function of the embryonic velum of two closely related species of *Crepidula* with different modes of development are examined. The velum of *C. dilatata*, a direct developer whose embryos feed on nurse eggs, does not differ substantially from the velum of *C. fecunda*, a species with planktotrophic larvae. Although velar ciliation develops earlier in embryos of *C. dilatata*, embryos of both species were able to feed on small particles, using the opposed-band ciliary mechanism. However, the embryos of *C. dilatata* lose this ability as they grow. The embryos of *C. dilatata* were not able to swim, whereas those of *C. fecunda* swam consistently in vials of seawater. This difference in swimming ability is probably due to differences in velum-body size allometry between the two species.

Introduction

In marine invertebrates, the evolution of direct development from the putative ancestral state of development with planktotrophic larvae is often accompanied by modifications of morphological structures associated with swimming and feeding (Strathmann, 1978; Hadfield and Iaea, 1989; Emlet, 1994; Byrne, 1995; Byrne and Cerra, 1996; Wray, 1996; Hadfield *et al.*, 1997). The functional modifications associated with the loss of feeding in pelagic echinoderm larvae are relatively well understood. For example, the ciliary bands that are arranged into complex loops along

larval arms in feeding larvae are reorganized into transverse bands or fields of cilia around a cylindrical body in nonfeeding larvae (Emlet, 1994). Among echinoderm larvae, such modifications are believed to maximize feeding efficiency in planktotrophic larvae and maximize swimming efficiency in nonfeeding planktonic larvae (Emlet, 1994). Modifications or loss of larval feeding or swimming structures are often so extreme that re-evolution of such structures appears to be difficult. Loss of these complex structures is cited as evidence that feeding larvae, once lost, cannot be regained (Strathmann, 1978). The morphological modifications associated with the loss of feeding larvae in other groups of marine invertebrates are less well understood. In marine gastropods, for example, the evolution of nonfeeding or direct development is usually associated with encapsulated development. The functional requirements of development within a capsule may be very different from the requirements of free-living development. For example, the embryo may need structures to circulate intracapsular fluid (Strathmann and Chaffee, 1984), ingest intracapsular fluid (Fioroni, 1966; Morrill, 1982; Rivest, 1992; Moran, 1999), or ingest nurse eggs or siblings (Rivest, 1981).

The morphological structure associated with both feeding and swimming in gastropod larvae is the velum. In planktotrophic larvae, the velum is normally a pair of large flat lobes of tissue edged with two opposed bands of compound cilia, extending from behind the head. The anterior band of cilia, the preoral band, and the posterior, postoral band flank a "food groove" lined with shorter cilia (Werner, 1955; Strathmann and Leise, 1979). Gastropods with planktotrophic larvae, and especially those with long-lived planktotrophic larvae, often have large elaborate vela with as many as six or eight lobes (Fretter and Pilkington, 1970; DiSalvo,
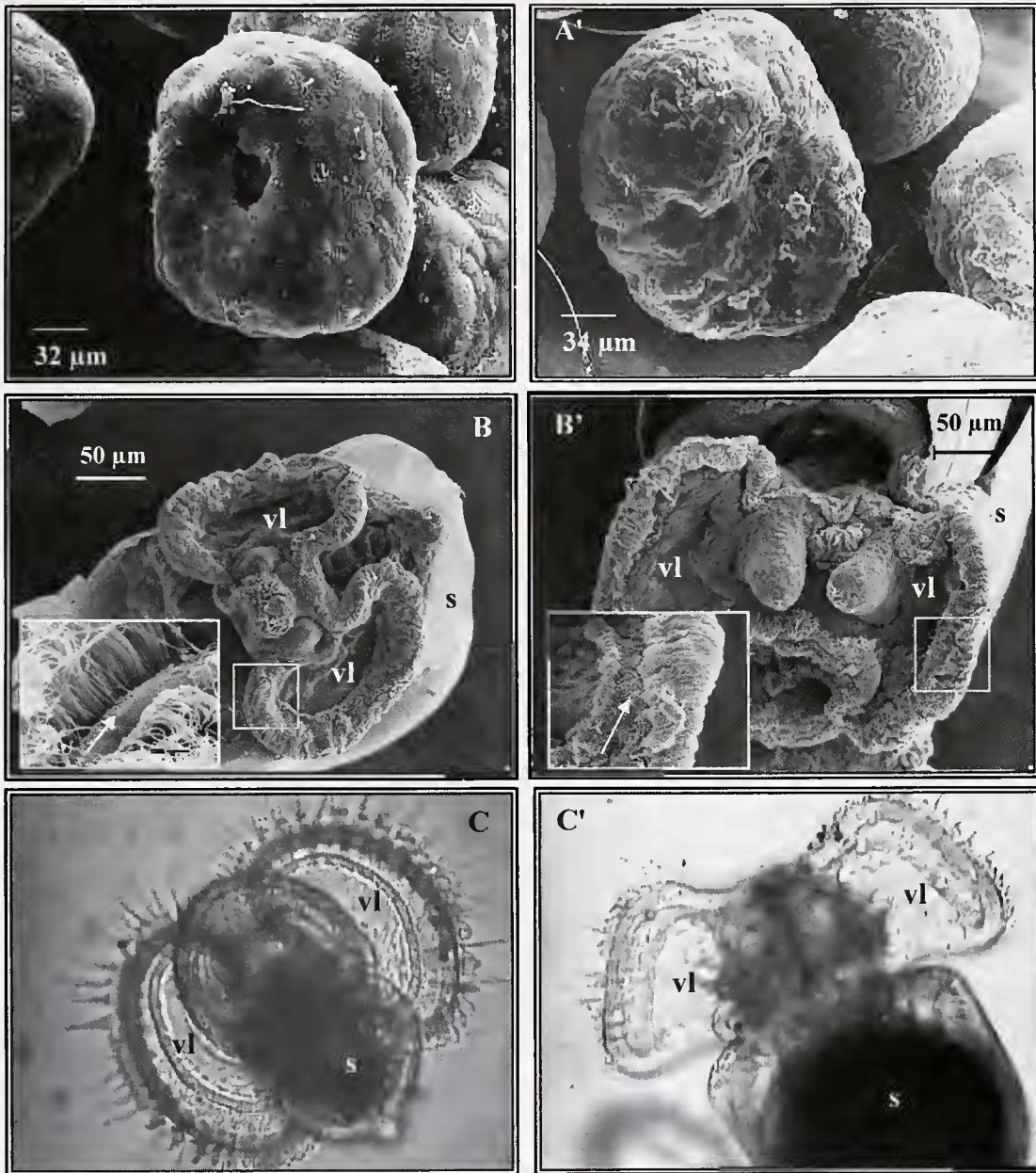
Figure 1. Scanning electron micrographs of different developmental stages of *Crepidula fecunda* (left side pictures: A–C) and *C. dilatata* (right side pictures: A′–C′). (A) Gastrula. (B) Intracapsular veliger; arrow in enlarged area indicates the food groove. (C) Light micrograph of the same stage as in B, showing the opposed-band ciliation and organized metachronal wave in both species.

1988). In larvae of *Crepidula fornicata*, the velar lobes grow significantly larger, in relation to shell size, when larvae are cultured with low concentrations of food rather than with high concentrations (Klinzing and Pechenik, 2000). In swimming larvae, the velum is lost at metamorphosis. Those species with abbreviated pelagic phases or complete intracapsular development often show a reduction in the size of the velum, loss of ciliary bands, and shortening of cilia (Fioroni, 1966). Hadfield *et al.* (1997) showed that

"archaeogastropod" larvae, which are characterized by swimming, nonfeeding larvae, have well developed preoral cilia but lack the food groove. In embryos of *Crepidula adunca*, a species with direct development from large yolky eggs, the velum is reduced to a small ridge with cilia only slightly longer than those on the surrounding mouth, tentacles, and head vesicle (Collin, 2000).

The absence of a feeding, swimming larval stage is not always associated with the loss of function of the velum. In

some species of *Littorina,* the embryonic velum is used to ingest albumin from the egg capsules (Moran, 1999). In these species the velum is as large, relative to embryonic body size, as the velum in species with planktotrophic larvae (Moran 1999), suggesting that this novel function is selecting against loss of structure and function of the velum. In embryos of *Petaloconchus montereyensis,* the velum is modified to ingest intracapsular yolk but has lost the preoral cilia. Other embryonic modifications associated with intracapsular development are reviewed in Fioroni (1966). In species with direct, nonswimming development, the velum is lost before hatching.

The present study was undertaken to examine structural and functional modifications of the embryonic velum associated with the evolution of nurse-egg feeding. We compare the embryonic development, especially the velar development, of two closely related species of gastropods with differing modes of development: *Crepidula fecunda* Gallardo 1979, a species with planktotrophic development, and *C. dilatata* Lamarck 1822, a species with direct development in which the embryos consume nurse eggs (Gallardo, 1976, 1977, 1979; Chaparro and Paschke, 1990). We measured the ability, relative to velar morphology, of the embryos to capture particles and to swim.

Materials and Methods

Adult *Crepidula dilatata* were collected subtidally in the estuary of Rio Quempillen at the north end of Chiloé Island, Chile (41°52′S, 73°44′W), and *C. fecunda* were collected from the intertidal of Bahía de Yaldad in the south of Chiloé Island (43°08′S, 73°44′W).

Prior to examination by electron microscopy, broods were removed from the females, individually incubated for 5 min in 0.5% solution of chloral hydrate, fixed in a 3% solution of gluteralderhyde with cacodylate buffer (pH = 7.4), and maintained at 4 °C for 1 h. Embryos were subsequently excapsulated, rinsed for 10 min in phosphate buffer, dehydrated in a graded acetone series, and critical-point dried. They were attached to stubs with double-sided tape, gold-coated, and examined with a Bausch & Lomb Nanolab 2000 scanning electron microscope.

To test for the ability of the embryos to feed on small particles, the embryos were excapsulated and placed in seawater at ambient temperature. Nontoxic red beads 2–10 μm in diameter (Chaparro *et al.,* 1993) were added to the solution. After 2 h the embryos were removed from the solution and fixed in formalin until they were analyzed. The presence of red particles in the larval or embryonic stomach was determined after fixation. One hundred embryos at each stage of development were examined under a compound microscope, and the percentage of embryos that had ingested particles (as indicated by red particles in the gut) was recorded. The method of particle capture was observed and
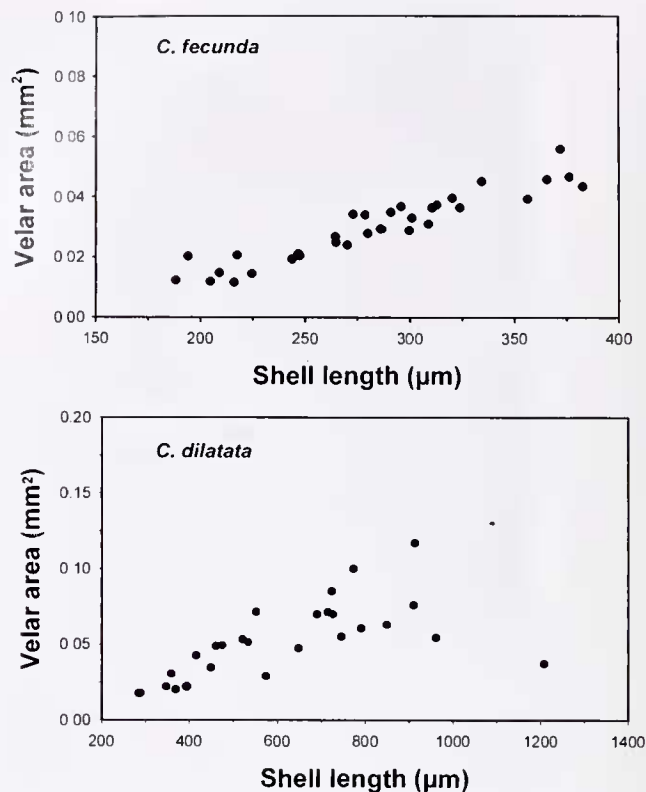

Figure 2. Scatter plot of velum area *versus* shell length for *Crepidula fecunda* and *C. dilatata.*

recorded using an inverted microscope connected to a Sony DXC-C1 video camera and a Sony PVM-1953MD monitor. Similarly, to test for swimming ability, embryos were excapsulated, and groups of 20 were placed in 20 ml of seawater. After 5 min, the number of embryos swimming above the bottom of the vial was recorded. The shell length and velar characteristics of embryos used in both experiments were measured, using Scion Image PC (Scion Corporation, Frederick, MD), from video images.

Results

Embryonic morphology

Embryos of both *Crepidula dilatata* and *C. fecunda* develop from eggs with average diameters of 250 and 212 μm respectively (Gallardo, 1977). However, the embryos of *C. dilatata* consume uncleaved nurse eggs (Gallardo, 1977) and hatch as crawling juveniles of about 1100–1200 μm shell length (Chaparro and Paschke, 1990), whereas those of *C. fecunda* do not consume nurse eggs, and they hatch as planktotrophic larvae at a size of 400–500 μm. Despite this difference in mode of development, the embryonic morphologies of both species are similar.

In both species, spiral cleavage and gastrulation by epiboly result in a very slightly flattened gastrula with a central
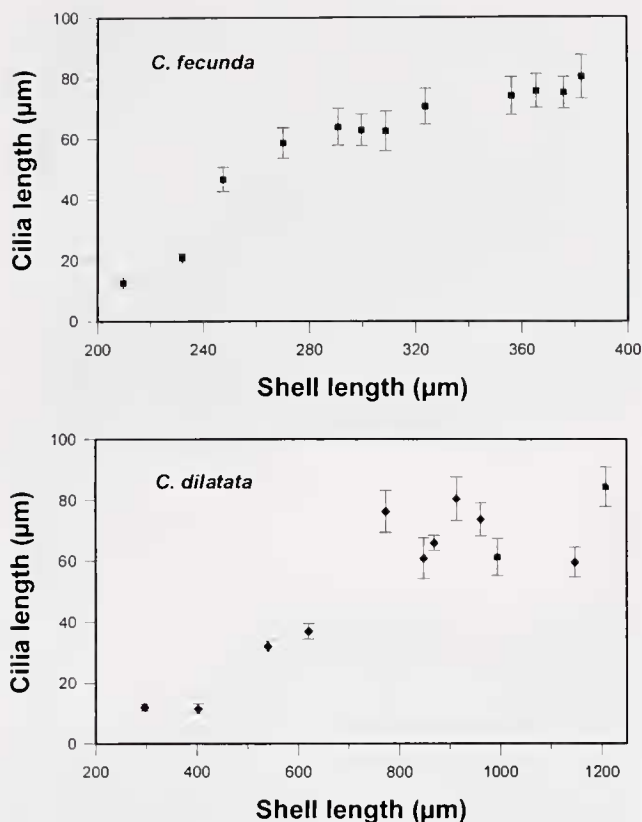
Figure 3. A comparison of cilia length *versus* shell length for *Crepidula fecunda* and *C. dilatata*. The vertical bars represent the standard deviation.

blastopore. In *C. dilatata* (Fig. 1A'), the gastrula is somewhat more elongate than the round gastrula of *C. fecunda* (Fig. 1A). The initial gastrula is not ciliated, but patches of cilia in the area of the presumptive velum and head vesicle can be seen in scanning electron micrographs of later gastrulas.

In both species, an intracapsular trochophore stage follows the gastrula. This stage is characterized by elongation of the embryo and flattening of the posterior dorsal area where the shell begins to form. In *C. dilatata*, clearly defined patches of cilia around the mouth, at the ventral anterior (presumptive head vesicle) and ventral posterior (telotroch) areas of the embryo, and lateral to the mouth (presumptive velum) begin to develop. In *C. fecunda*, these cilia are less obvious, and their elaboration occurs later, relative to other morphological events, than in the embryos of *C. dilatata*.

As the edge of the velum extends laterally from the body wall, the distinct bands of cilia can be observed along its edge in *C. dilatata*. It is at this stage that the preoral cilia first appear in *C. fecunda*; the postoral and oral cilia appear slightly later. At this stage, in both species, the embryonic kidney is clearly visible on each side of the embryo, the shell begins to grow around the posterior end of the embryo,

and the foot begins to differentiate and becomes ciliated. In embryos of both species of 200–300 μm, the small head vesicle is distinct and covered with cilia, the velum clearly shows opposed band ciliation, and the foot has an operculum and a medial band of ventral cilia (Fig. 1B, B', 1C, C'). Subsequent development reflects the growth and elaboration of these structures. The shell grows to contain the yolk and developing viscera. The lobes of the velum grow wider, and the preoral cilia grow to a length of 80 μm. Larvae of *C. fecunda* hatch at a shell length of 400–500 μm, after the reabsorption of the embryonic kidneys and the head vesicle.

In *C. dilatata*, further morphogenesis occurs in the capsule. The tentacles begin to form at a shell length of about 500 μm. Both tentacles are large and the apical ciliary tufts are well developed, as is the foot, in embryos with a shell length of 700 μm. At this stage the velar cilia reach their maximal length of 80 μm. Embryos between 800 and 1100 μm in shell length undergo intracapsular metamorphosis. The pedal glands become visible, and the velum gradually shrinks. By the time the embryo is about 1000 μm in shell length, the velum and embryonic kidneys are no longer visible. This is also near the length at which the embryos usually exhaust the supply of nurse eggs (Chaparro and Paschke, 1990). Among advanced embryos, the shell length
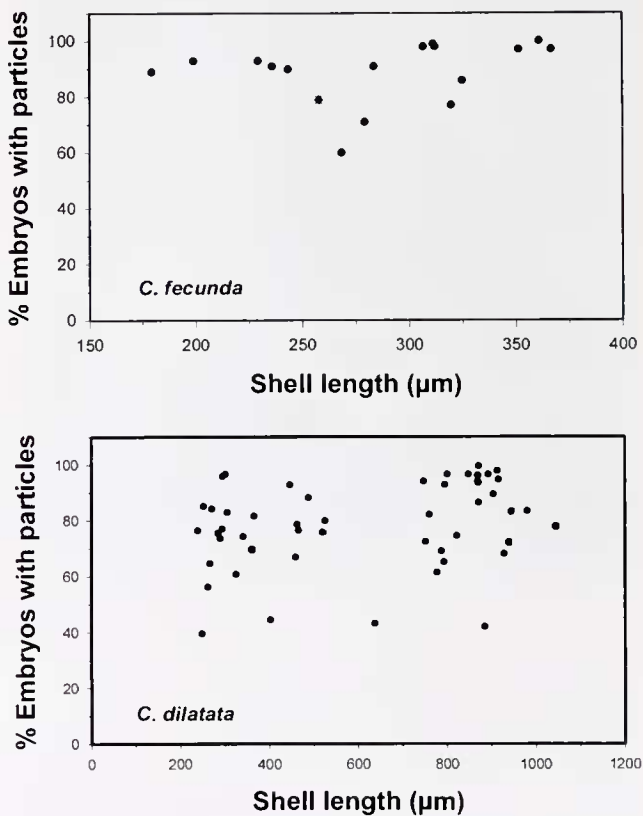

Figure 4. Scatter plot of the proportion of embryos that ingested particles *versus* shell length for *Crepidula fecunda* and *C. dilatata*. Each point represents a trial with 100 embryos.
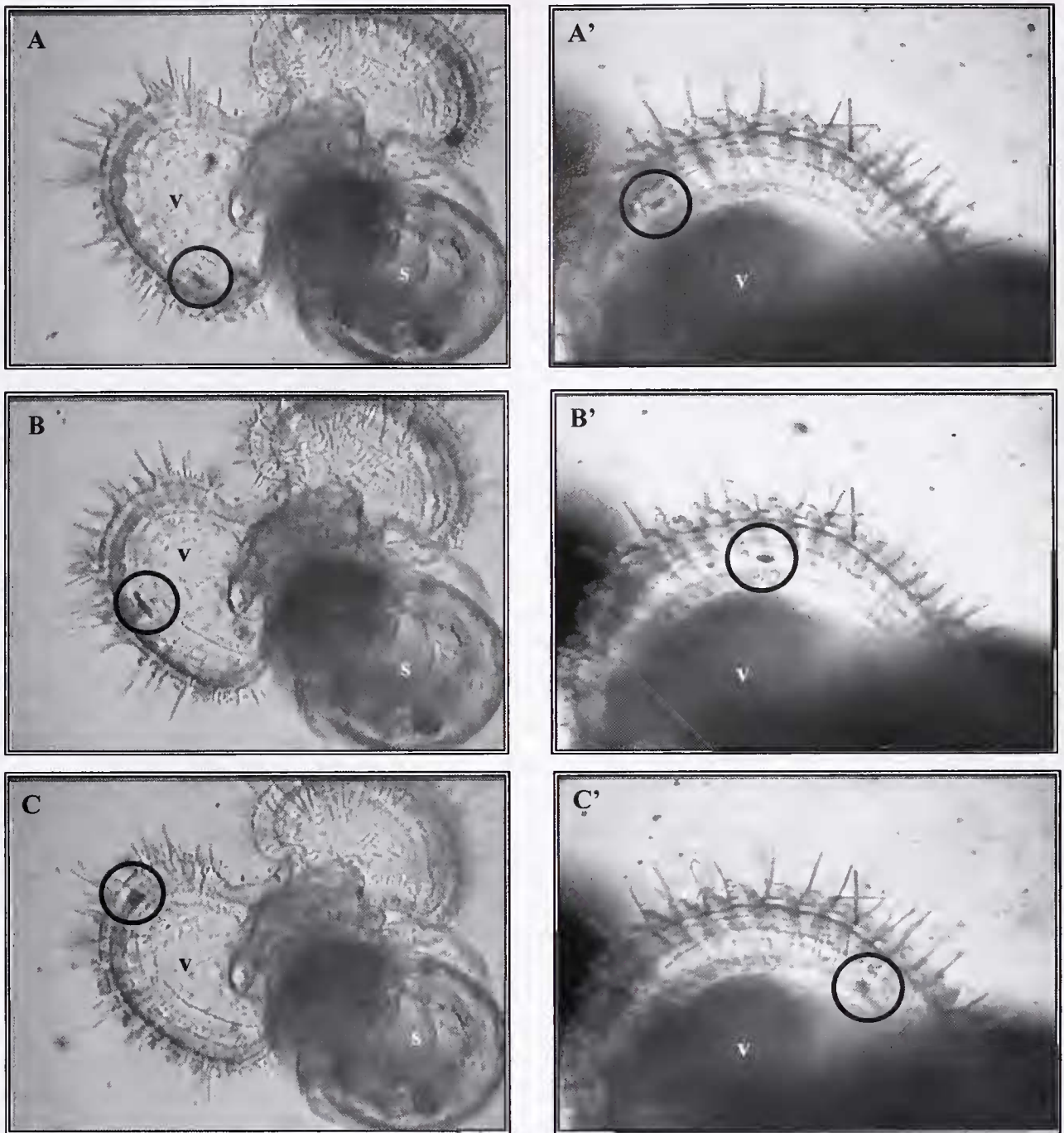
Figure 5. Video sequence of a captured particle (encircled) moving along the food groove towards the mouth. Left side (A–C): *Crepidula fecunda* embryo. Right side (A'–C'): *C. dilatata* embryo.

at any given developmental stage shows variation due to differences in the number of nurse eggs consumed by each embryo.

Allometry

In *C. fecunda*, the velum grows throughout intracapsular development. The velar area increases linearly with respect to shell length (Fig. 2), and it reaches its maximal size, about 0.06 mm², near hatching. The size of the velum relative to shell length in *C. dilatata* is initially the same as in *C. fecunda*, but the velum grows more slowly relative to shell length. Comparisons of larvae at a comparable shell length (<600 μm) showed a significant difference in velum size relative to shell length (ANCOVA, $n = 374$; $f =$
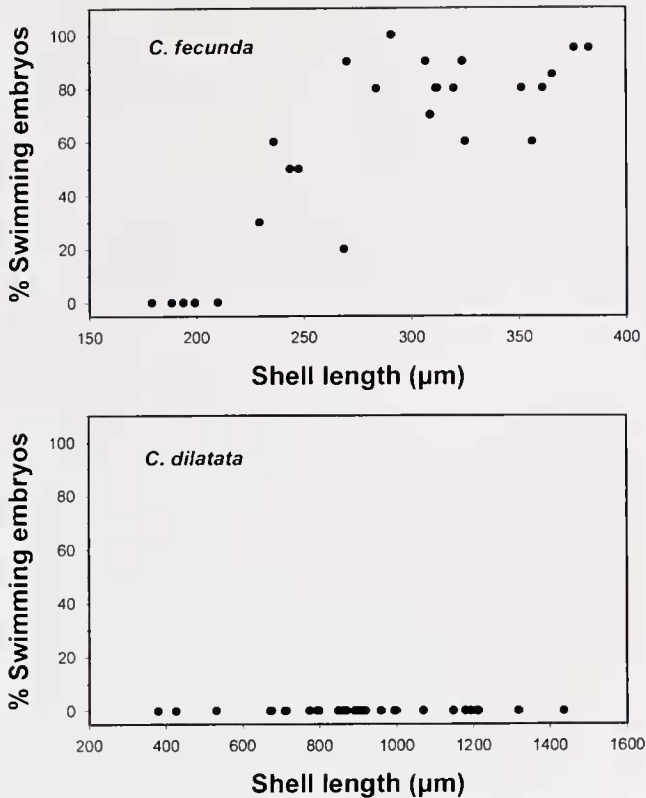
Figure 6. Scatter plot of the number of embryos that showed swimming ability *versus* shell length for *Crepidula fecunda* and *C. dilatata*.

227.987, $P < 0.001$). The velum in *C. dilatata* continues to grow until it reaches an area of about 0.12 mm². At large shell sizes the velar area decreases as the velum is absorbed prior to hatching.

The preoral cilia grow to a length of about 80–90 μm in embryos of both species (Fig. 3), but the relationship between the lengths of cilia and shell differs between the two species. In *C. dilatata*, the direct developer, the cilia reach their maximal length at a shell length of approximately 700 μm (near the size of maximal velar area); the same maximal length is attained in *C. fecunda* at a shell length of about 320 μm. The velar cilia of *C. fecunda* continue growing until hatching, as does the velum.

Feeding and swimming

The embryos of both *C. dilatata* and *C. fecunda* were able to capture and ingest small particles from suspension in seawater (Fig. 4). A high percentage of *C. fecunda* embryos from a shell length of 175 μm to the hatching size of 350–450 μm showed the ability to capture and ingest beads. In *C. dilatata*, the small embryos (shell length less than 400 μm) ingested particles with high frequency. Observation of both species showed that the embryos use the opposed-band ciliary mechanism to capture particles and to

move them along the food groove to the mouth (Fig. 5A, A′, 5B, B′, 5C, C′).

There was a striking difference between the species in swimming ability: embryos of *C. dilatata* never swam, whereas embryos of *C. fecunda* larger than 270 μm swam consistently in our experiment (Fig. 6).

Discussion

Our observations of *Crepidula* embryos show that the embryonic morphology of the direct-developing *C. dilatata* is little modified from that of the planktotrophic *C. fecunda*. After consuming many nurse eggs, the embryos of *C. dilatata* are larger than those of *C. fecunda*, and the relative size of the velum is smaller; however, the operculum is not lost, and there is no elaboration of the embryonic kidneys or massive enlargement of the head vesicle as has been reported for embryos of another direct-developing *Crepidula* species (Moritz, 1939; Collin, 2000). The velum of *C. dilatata* is not lost or greatly reduced in absolute size as it is in many gastropods with direct development, and it appears to be structurally similar to the velum of the closely related planktotrophic *C. fecunda*. It retains the bands of both preoral and postoral cilia and has a ciliated food groove. In addition, *C. dilatata* retains the ability to use the opposed-band ciliation to capture and transport particles along the food groove.

The lack of change in the embryonic development in the direct-developing *C. dilatata* could be the result of several factors. It is possible that there are few overall modifications of development because direct development has evolved recently in this species. Gallardo (1979) suggested that *C. fecunda* and *C. dilatata* were recently diverged sibling species The retention of a well-developed velum with a functional opposed-band ciliary mechanism in direct developing species is unusual in North American *Crepidula* that do not consume nurse eggs (RC, pers. obs.) It is probable that the continued use of this structure to feed on material within the capsule has maintained the velum as a functional structure. Small particles of yolk from abnormal or damaged embryos pass along the velum of young embryos of *C. dilatata* in the same manner as the artificial particles described here. Nurse eggs are ingested in a different manner. In *C. dilatata*, the nurse eggs are generally consumed after the embryos have reached a shell length of 500 μm (Chaparro and Paschke, 1990) and have developed a large ciliated velum (see above). The embryo rotates the nurse egg on the velum. The egg deforms as the cilia of the buccal region slowly draw the egg into the esophagus (OC and RC, pers. obs.). The ability of *C. dilatata* embryos to capture small particles before the stage at which they actively feed on nurse eggs suggests that small particles from damaged embryos may be an important source of early nutrition in young embryos.

The energetic contributions of these different feeding mechanisms should be examined in greater detail.

A previous study that addressed the possibility of the re-evolution of feeding gastropod larvae concluded that *Petaloconchus montereyensis* has lost the velar structures necessary for swimming and feeding, making the re-evolution of feeding larvae that use the opposed-band ciliary mechanism on the velum unlikely (Hadfield and Iaea, 1989). Because, as demonstrated here, *C. dilatata* embryos retain the ability to capture particles by using the opposed-band mechanism, the re-evolution of feeding larvae from this species is not impossible. However, changes in the velum-body size allometry and hatching size would probably be necessary to produce a larva capable of swimming. Because the hatching size for *C. dilatata* is determined by the number of nurse eggs per embryo in each capsule, it is possible that velum-body size allometry is determined by the number of nurse eggs consumed. If this is the case, a drastic reduction in the number of nurse eggs per capsule could change this allometry enough to produce embryos capable of swimming.

Acknowledgments

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